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NUTRITION REVIEWS

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NUTRITION REVIEWS

VOL. 16 JULY 1958 No. 7

INTERDEPARTMENTAL COMMITTEE ON NUTRITION FOR NATIONAL DEFENSE

During the past few years our Government has recognized the importance of food and nutrition as an integral part of its mutual assistance programs of technical, military and economic aid to the developing friendly countries. The Interdepartmental Committee on Nutrition for National Defense (ICNND) was formed as a result of the recognition of the importance of nutrition and of the lack of personnel with experience in public health, nutrition and agriculture to cope with such a coordinated activity. These points were emphasized by the results from nutrition surveys conducted by the U. S. Armed Forces in the Republic of Korea and the Republic of China (Taiwan) in 1953–1954. These surveys revealed that much of the data in reference to food and nutrition which had been collected was not generally available to the groups having operating responsibilities in this area. In one country no less than six different groups were independently making nutrition studies. A coordination of these studies was indicated to effect economy and to consolidate the recommendations.

In view of the interest of numerous Government agencies, an ad hoc committee was established in the summer of 1954 upon the suggestion of Dr. Frank B. Berry, Assistant Secretary of Defense (Health and Medical), Dr. W. H. Sebrell, Jr., former director of the National Institutes of Health, Dr. S. Bayne-Jones, Office of the Surgeon General, Department of the Army, Dr. Howard T. Karsner, Office of the Surgeon General, Department of the Navy, and the late Dr. Harold R. Sandstead of the U. S. Public Health Service. The Committee was formally constituted with Executive approval early in 1955 and a memorandum of agreement was signed by the Secretaries of the interested agencies: Departments of Defense, State, Agriculture, Health, Education and Welfare and the International Cooperation Administration. The Committee was later expanded to include the Atomic Energy Commission.

Committee membership includes representatives from the above agencies, with the Department of Defense also being represented by members from the Army, Navy and Air Force. Dr. Berry serves as Chairman of the Committee, and a Secretariat, consisting of an executive director, a clinician, a nutritionist and an agricultural economist, is located at the National Institutes of Health. A panel of approximately twenty consultants who are specialists in the fields of nutrition, medicine, biochemistry, food technology and agriculture, all with experience in the developing areas of the world, serves as an advisory body to the Committee.

The primary function of the Committee is to coordinate nutrition programs of the United States agencies operating in foreign countries and to give support to our military and technical missions. The Committee serves as a central clearinghouse on food and nutrition information, reviews nutrition projects being conducted in special areas and, when appropriate, coordinates, advises and participates in field studies.

Following a visit to a number of countries in the Near East by Dr. Berry and Dr. Sandstead in 1955, and because of the interest of the countries in nutrition, the Committee received requests for assistance in conducting nutrition surveys and in establishing nutrition services from Iran, Pakistan, Turkey, the Philippines, Korea and Libya. In January 1956, as a part of the Mutual Defense Assistance Program, the United States in-

augurated the nutrition program and nutrition surveys have now been completed in these six countries.

The objectives of the surveys are primarily to assess, to assist and to learn. The assessment phase involves an evaluation of the nutritional status of the population, which defines the major problems and serves as a basis for practical recommendations for improvement of nutrition and health. In conjunction with the surveys, assistance is given (1) through the training of counterpart host country personnel in nutrition evaluation techniques, emphasizing clinical and biochemical evaluation, dietary intake and food production and processing; (2) by furnishing essential laboratory equipment and supplies for establishing a medical nutrition and food laboratory; (3) by practical recommendations so that the host country can best utilize its own resources to help itself. The surveys afford an unusual learning opportunity for U. S. personnel regarding the nutritional diseases, the foods, food patterns, habits, customs, practices and research. The survey experiences are serving as a remarkable stimulus to clinical nutrition and research in both the United States and the host countries.

A project is developed as follows. Upon request for assistance from a country receiving Mutual Defense Assistance, and after approval by the ICNND, a nutrition survey team is organized by appointing specialists in the fields of medicine, nutrition, sanitation, food technology and agriculture. The U. S. nutrition team members include a survey director, one or two clinicians, a laboratory director and two assistants, two food and dietary survey specialists (usually former U. S. Army Nutrition Officers), a food technologist, an agricultural economist and a sanitary engineer. The wholehearted support of our universities, colleges, armed services, the Army Medical Nutrition Laboratory and the National Institutes of Health in releasing personnel for these studies is most encouraging. The host country assigns counterpart personnel, furnishes laboratory space, transportation within the country and other logistical support. The equipment supplied by the United States is given to the host government upon completion of the survey. The duration of each survey is approximately 70 to 90 days.

A "Manual for Nutrition Surveys" has been prepared by the ICNND, its consultants and team members, to serve as a detailed guide for the conduct of nutrition surveys. It includes directions for all aspects of the nutrition survey (*i.e.*, clinical, laboratory, dietary, sampling considerations, and the like) as well as forms for records and suggested standards of interpretation of data. The Manual incorporates the experiences of the first four field studies, and is available at the Government Printing Office.

This Manual will contribute greatly to the establishment of uniformity in methods, techniques and procedures, and thus will facilitate a reliable comparison of results of surveys within and among countries. It will serve as a reference to insure maximum coverage of the major points considered essential in appraising nutritional status to permit practical, effective recommendations which are consistent with the countries' resources and customs; it will serve as a guide in defining the responsibilities and duties of various team members, and it will assist in training counterpart personnel in survey techniques and in establishing permanent nutrition services.

The clinical team conducts a physical examination on a statistical sample, the exact number being determined by the population size. In general, approximately 2,000 individuals are given a detailed examination and, in addition, 3,000 to 5,000 individuals are given a less extensive examination in which definite indicator lesions of nutritional deficiency are recorded. Samples of urine and blood are obtained from approximately 500 of the subjects examined in detail. The biochemical analyses include determination of hemoglobin, hematocrit, plasma protein,

vitamin C, carotene and vitamin A, urinary thiamine, riboflavin and N′methylnicotinamide. Should time permit or findings so dictate, the biochemical team is equipped to determine total serum cholesterol, serum albumin and globulin, and to make vitamin saturation tests.

The daily food intake is determined by inventory and food preparation survey techniques and, in addition, composite food samples are collected for chemical analysis. Data are obtained on menu planning and food issues, habits, production, preparation, processing, storage and transportation.

Detailed findings of the surveys will be published in scientific journals in the near future. In general, the nutritional status of the Armed Forces of Iran, Turkey, the Philippines, Korea and Pakistan was reasonably good. The surveys, however, identify in each country improvements which can be made in the nutrition. The major nutritional needs concern improvement in riboflavin and, in some areas, vitamins A and C. The incidence of borderline nutritional deficiency symptoms varied greatly between areas within each country. For example, in one country the per cent of individuals with angular lesions and low urinary riboflavin excretion (less than 30 mcg. per six-hour fasting sample) ranged from 7.5 to 19.0 with lesions and 4.5 to 30.0 with low riboflavin excretion. The per cent of examinees with plasma vitamin C levels less than 0.1 mg. per 100 ml. ranged as high as 94.0 in an area where the incidence of scorbutic gums was 3 per cent and where approximately 40 per cent of the examinees had bleeding gums and marginal swelling. With reference to vitamin A, in one area 68 per cent of the troops had plasma levels of less than 20 mcg. per 100 ml. in association with an approximate 10 per cent incidence of xerosis and 46 per cent follicular keratosis.

The principal recommendations have dealt with improvement of ration planning and of distribution of food, and the establishment of a nutrition service which can be used as a focal point for nutrition programs and education. Recommendations have often involved the eventual goal of developing food processing, preservation and storage. In most instances the nutritional needs can be met through improved utilization of existing resources. The recommendations are made with due regard to the habits and customs in the country.

Data are now available on the clinical examination of over 30,000 individuals, 4,000 urine and blood analyses and dietary intake studies, representing over a million man days. These data will allow study of methodology as well as give evidence such as that discussed above. An example of one of the basic problems of methodology encountered is the interpretation of urinary riboflavin excretion values in populations subsisting on a borderline to low intake of thiamine and riboflavin. Clinical and dietary evidence of riboflavin deficiency was present; however, urinary excretion of riboflavin was at a level where one would normally not expect clinical findings.

The first opportunity to evaluate the influence of recommendations based on a nutrition survey came in 1956 when a resurvey was made of the Korean Armed Forces after an initial survey in 1953 by the U. S. Army. The more recent survey indicated that there was a vast improvement in the nutritional health of the troops as compared to their status in 1953. Many of the recommendations made in 1953 to improve ration planning, purchase and distribution had been implemented and were largely responsible for the great improvement in the health of the troops. In the 1956 survey only 16 per cent of the troops examined were under 90 per cent standard weight (based on age, height and weight, U. S. Medico-Actuarial Reference Tables) as compared to 46 per cent in 1953. In 1953 the incidence of angular lesions in recruits completing a 16-week training period was 45 per cent, versus 28 per cent in 1956. In 1953 the incidence in the same troops of calf

tenderness, Bitot's spots and scorbutic gums was 3.6, 5.5, and 45.9, respectively; in 1956 these lesions were not encountered.

As a result of contacts made by the U. S. nutrition survey teams in Iran and Pakistan, the Iranian Government invited members of the Baghdad Pact to a general nutrition conference in Tehran in November 1956. The conference was attended by representatives of the Armed Forces of Iraq, Pakistan, Turkey, the United Kingdom and the United States. The delegates passed a resolution recommending that an Internation Committee on Nutrition be organized on a permanent basis, with meetings to be held annually to discuss mutual nutrition problems. The delegates also requested the Interdepartmental Committee on Nutrition for National Defense to serve as the secretariat for the first year of operation. The U. S. Government, through the Departments of State and Defense, has authorized U. S. participation in the Internation Committee. The second meeting of the Committee was held in Ankara, Turkey, in April 1958.

The Interdepartmental Committee on Nutrition for National Defense now plans to have consultant groups review the data accumulated during the past six surveys, with the view of assessing present concepts of clinical nutrition and nutritional requirements. One of the immediate problems is to re-evaluate the criteria now employed in diagnosing nutritional diseases, especially those resulting from multiple causes, and in determining borderline or suboptimal nutrition.

Nutrition surveys in the developing countries afford an opportunity for experience and training of personnel of the host country and of the U. S. team in a wide variety of nutritional disease patterns. Concurrent with present-day interest in the possible relationship of diet to heart disease, dental health, cancer, etc., these surveys present an excellent research opportunity to study these problems. The program of the Committee anticipates conducting three nutrition surveys per year, with a continued follow-up in the countries previously surveyed.

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NUTRITION AND NITROGEN METABOLISM IN THE SURGICAL PATIENT

The many attempts made to reverse the negative nitrogen balance, which usually follows surgery, have stimulated the development of high quality protein hydrolysates, fat emulsions, and carbohydrate solutions. In addition, an understanding of the essential role of electrolytes and the anabolic action of androgens has made possible a more reasonable supplementation of the inadequate diet intakes characteristic of ill patients, or patients unable to eat as a result of gastrointestinal surgery.

The belief that nitrogen losses are intractable during the first few days following surgery has led to the description of this interval as the period of rejection of nitrogen (F. D. Moore and M. R. Ball, *The Metabolic Response to Surgery, Charles C. Thomas, Springfield, Ill. (1952)*). Failure to reverse the negative nitrogen balance of surgical patients using a protein hydrolysate led A. W. Wilkinson, B. H. Billing, G. Nagy and C. P. Stewart (*Lancet*, **533** (*1950*)) to the conclusion that nitrogen loss was inevitable during the early postoperative days. Unfortunately, the quantities of amino acids and calories which they gave parenterally would not be considered sufficient to maintain adequate nutrition in the post-surgical patient.

Evidence that postoperative nitrogen deficits can be minimized, or prevented, has

been summarized in the report of W. D. Holden, H. Krieger, S. Levey and W. E. Abbott (*Ann. Surg.* **146,** *563 (1957)*). Patients or volunteers, fed either orally or intravenously, were studied with regard to nitrogen and electrolyte balances. For comparison, male volunteers underwent a period of starvation for four days during which there was weight loss and a nitrogen deficit of about 12 g. daily. Similar volunteers getting a low calorie, low protein diet (10 cal. per kg. and 0.54 g. N per kg. of body weight) lost similar amounts of weight and showed similar deficits of nitrogen (about 7 g. per day). Male patients experiencing minimal operative trauma receiving a 9 calorie per kg. diet and no nitrogen, showed postoperative deficits of nitrogen of about 8 g. daily. When appendectomy was uncomplicated, the metabolic balance determination showed about 7.5 g. of nitrogen deficit when nitrogen was not given. When appendectomy was complicated by peritonitis, the nitrogen deficit became 15 g. daily.

In contrast to the males, similar studies on two female patients receiving no nitrogen and one normal female volunteer receiving low calorie, low nitrogen alimentation, showed much smaller nitrogen deficits during the balance periods. The normal subject had a deficit of about 5 g. of nitrogen daily. The patient with uncomplicated appendicitis showed an average loss of 6.4 g. per day, and the patient whose appendectomy was complicated by peritonitis showed an average loss of 10.6 g. nitrogen daily.

Comparison of an intravenous feeding regimen of dextrose only, with one in which a protein hydrolysate was offered, was made possible when a patient underwent plication of gastric ulcer, and later elected to have subtotal gastrectomy. During the first moderately simple operation, 3 liters of 5 per cent dextrose were given daily and the nitrogen deficit for four and one-half days was 45 g. Two months later the second operation was performed but 2 liters of 5 per cent protein hydrolysate containing 5 per cent dextrose, and 1 liter of 10 per cent dextrose solution, were given daily. At this time the five-day cumulative nitrogen deficit was only 9 g.

Of a group of 33 patients having uncomplicated subtotal gastric resection, 14 were given 3 liters of 10 per cent dextrose daily and a representative member of this group showed a 61 g. nitrogen deficit during five postoperative days. Seven patients received 1.5 liters of 5 per cent protein hydrolysate containing 5 per cent dextrose and 2.2 liters of 10 per cent dextrose. A representative of this group had a cumulative nitrogen deficit of 39 g. in five days after surgery. A third group of 12 patients received 3 liters of 5 per cent protein hydrolysate containing 10 per cent fructose and 600 ml. of a 15 per cent fat emulsion. The cumulative five-day nitrogen deficit was 3 g.

The effect of a long acting anabolic hormone was tried in one patient getting 25 calories and 0.2 g. of nitrogen per kg. per day following hemigastrectomy. The five-day nitrogen deficit was only 15 g. A female patient given 250 mg. of the long-acting anabolic steroid before subtotal gastric resection, and receiving 28 calories and 0.25 g. of nitrogen per kg. per day postoperatively, showed a five-day cumulative positive nitrogen balance of 2.8 g.

These studies indicate that females show a smaller nitrogen deficit than do males undergoing similar operations and receiving similar diets, that nitrogen losses can be materially reduced in the early postoperative period by giving adequate amounts of calories and nitrogen, and that long acting anabolic steroids given along with moderate calorie and nitrogen feeding promote greater retention of nitrogen. Although it is possible for patients to be deprived of adequate protein intake for a few days without permanent ill effects, it is not known whether this period of deprivation significantly alters the patient's rate of recovery. There is little reason to believe that fasting a traumatized patient would be beneficial, particularly in patients who may have some depletion of their tissues before surgery.

THIAMINE AND RIBOFLAVIN EXCRETION IN CHILDREN

One technique in the assessment of thiamine and riboflavin nutriture is the measurement of the 24-hour urinary excretion of these vitamins. G. Stearns, *et al.*, (*Am. J. Dis. Child.* **95,** *185 (1958)*) have measured the 24-hour thiamine and riboflavin excretion in normal children. Eighty-four six-day studies and 45 four-day studies were carried out on 21 children, 5 girls and 16 boys ranging in age from 4¾ to 13 years and in weight from 16 to 42 kg. The children were on diets considered as adequate before the study began and were not saturated with vitamins. Each child received a basal diet, which was adequate in all known nutrients, and two experimental diets wherein one food was given at two different levels varying from 3 to 33 per cent of the total solids ingested. Both basal and experimental diets were fed for a period of three days before collections were begun and for an experimental period of six days. Data were presented which supported the validity of using a conditioning period as short as three days in the thiamine studies, since the changes in intake were followed by consistent changes in excretion.

When the results were expressed as per cent of intake there was considerable scatter. At intakes greater than 1.5 mg. the urinary thiamine varied from 11 to 44 per cent of the intake, but the majority of values exceeded 20 per cent. At intake levels between 0.7 and 1.4 mg. daily, urinary excretion varied from 10 to 36 per cent of the intake, with the mean value approximating 20 per cent. All of the subjects in this study having intakes less than 0.4 mg. per day excreted less than 10 per cent of the thiamine intake. When the results were expressed as milligrams per gram of creatinine, it was noted that 72 out of the 84 studies showed urinary thiamine excretions greater than 0.25 mg. per gram of creatinine. Nine of the children excreted between 0.15 and 0.25 mg. per gram of creatinine. In only three was the urinary thiamine less than 0.15 mg. per gram of creatinine.

Six children were given diets containing 0.326 or 0.345 mg. of thiamine daily. These subjects excreted less than 0.04 mg. daily within six days. After 18 days the subjects were repleted by returning to an adequate diet, and achieved thiamine excretions of greater than 0.10 mg. per day within four days. However the children were still quite listless and they were further repleted with large doses of thiamine. During this part of the study the urine thiamine excretion decreased to 4 to 9 per cent of the intake, showing excellent conservation. Clinical signs of deficiency, especially listlessness, appeared early and leg cramps appeared in one patient at the time that the urinary thiamine excretion fell below 0.03 mg. per day.

Fecal thiamine was also measured and was more variable than the urinary excretion. The difference between intake and excretion varied from −0.01 mg. to a maximum 1.60 mg. on the highest intake. In ten studies the differences were from 0.70 to 0.79 mg. daily. During the studies at very low thiamine intakes, the fecal thiamine did not vary consistently in either direction. In neither the adequate nor the low intake periods did the values for fecal excretion warrant the assumption of any demonstrable amount of absorption of thiamine synthesized by intestinal bacteria.

Studies on the excretion of riboflavin were carried out on all of these same subjects. The riboflavin intake ranged from 0.79 to 2.70 mg. per day and the excretion was 33 to 70 per cent of the intake. The riboflavin data were presented also as micrograms per kg. per day, with the intake ranging from 35 to 120 and the excretion varying between 18 and 60. The correlation coefficient between intake and excretion was high, 0.85 on the per diem and 0.82 on the body weight basis. There was so little relationship to the

caloric intake that the correlation was not calculated.

As with thiamine, the intake appeared to be the main factor in the determination of excretion. The changes in nitrogen balance in these subjects were relatively small and no correlation with riboflavin excretion was apparent. Riboflavin excretion amounted to between 0.829 and 3.382 mg. per gram of creatinine, a high value by customary standards. There was no evidence that the period of relative thiamine deprivation had any effect upon the riboflavin excretion.

In three subjects the riboflavin intake was decreased from 55 to 101 micrograms per kg. per day to 20, and the thiamine from 25 to 32 to 10 to 12. While the thiamine excretion decreased immediately, both in absolute amounts and as per cent of the intake, the riboflavin excretion exceeded 100 per cent of the lowered intake for several days and the absolute excretion decreased more slowly than did that for thiamine. Although this small study was terminated after nine days because of unpalatibility, the results suggest that a longer period of adjustment is needed in studies with riboflavin, or that these particular subjects had relatively greater stores of this vitamin. The contrasting pictures certainly indicate that the percentage of intake of water soluble vitamins appearing in the urine may depend upon the customary intake as well as on the intake at any particular time.

The children in this study had thiamine and riboflavin intakes characteristic of this age group in the United States. The observations on thiamine would suggest that the storage that was noted to occur in the studies on intakes above 0.4 mg. daily was not great enough to prevent depletion within ten days on intakes of 0.35 mg. daily. The high degree of correlation between intake and urinary excretion, and the rather rapid adjustment of excretion following changes in intake, would suggest that the 24-hour urinary thiamine excretion would reflect the adequacy of the intake. The data suggest that the excretion of less than 10 per cent of the intake in this age group was evidence of inadequate intake for both thiamine and riboflavin. The data for riboflavin indicate a very luxurious intake and considerable storage. The depletion studies on riboflavin were not as detailed or as informative as those for thiamine.

BODY WATER DETERMINATIONS IN UNDERNOURISHED INDIVIDUALS WITH EDEMA

The determination of the total water content in human subjects has been carried out using the dilution technique with various substances as the indicator (deuterium, tritium, antipyrine and urea). Since urea is distributed through the entire body water space, and is both non-toxic and easily determined, it possesses many attributes as a test agent for the determination of the water content in the intact individual.

There are, however, two questions inherent in a method using urea. Is the rate of urea formation in the individual constant during the period of study, and does the diuresis, which is induced by the ingestion of large amounts of urea, affect the body water determination significantly? In the case of normal individuals it has been shown that the total body water content, as determined by the urea method, agrees with the values obtained using deuterium. Such studies indicate that the urea method is suitable for the determination of total body water in normal individuals. However, two reports (C. Gopalan, *et al. Indian J. Med. Sci.* **6,** *277 (1952)*; E. G. Homes, E. R. Jones and M. W. Stanier, *Brit. J. Nutrition* **8,** *173 (1954)*) have indicated that when the urea method is used in severely malnourished edematous patients, excessively high levels of body

water were found (*e.g.* 87 to 90 per cent of the body weight). The possibility was suggested that in the severely protein depleted individual, urea nitrogen might be used as a non-specific source of nitrogen by the body tissues. This would account for the falsely high values for total body water noted.

In order to test the validity of the urea method for estimating the total body water in malnourished patients with edema, S. G. Srikantia and C. Gopalan (*Lancet* **II,** *1037 (1957)*) conducted studies in which the total body water was determined simultaneously by both the antipyrine and urea methods in five normal and five malnourished patients with edema. In the case of the normal individuals, blood samples were drawn two, three and one-half, and five hours after receiving the test compounds. The malnourished patients had blood samples removed at five, seven, and eight hours after receiving the antipyrine and the urea. The longer times in the edematous patients were necessary so the test substance could reach equilibrium throughout the entire body water. For both methods the total body water was expressed as the per cent of the body weight. Using the urea method it was found that the average body water for the five normal individuals was 58.4 per cent (52.6 to 61.4) while in the same patients the average total body water as determined by the antipyrine procedure was 57.9 per cent (48.8 to 65.2).

A comparison between the two techniques was done by calculating the urea:antipyrine ratio × 100 for the normal individuals, and this ratio averaged 101.3 (94.4 to 108.0). Thus, in the case of the normal individuals, these two methods seem comparable even though the total body water was slightly higher in three of the five individuals when determined by the urea method.

When applied to the malnourished edematous patients it was noted that the total body water, as determined by the urea method, averaged 75.8 per cent (65.1 to 89.3). The body water simultaneously determined by the antipyrine method had an identical average of 75.8 per cent (64.5 to 86.3). The urea:antipyrine ratio × 100 for this group of patients averaged 101.4 (95.3 to 104.4).

While the average values for total body water in the malnourished patients were identical as determined by both methods, it should be noted that four of the five patients had slightly higher total body water content when determined by the urea procedure. While the urea method shows individual values which may be slightly higher than those obtained using the antipyrine method, this difference occurs in both the normal subject and the malnourished patient with edema.

These investigators have concluded that the urea method is a simple and reliable technique for the determination of total body water, and that it can be applied to both the severely malnourished patient and normal individuals.

PENTOSE METABOLISM IN MAN

Recognition of a need for an understanding of the role of penetration phenomena in the metabolism of the muscle cell has resulted from the demonstration that the muscle cell membrane is responsive to insulin addition and deprivation, is a barrier to the free diffusion of sugars, and is, under certain conditions, the rate-limiting step in glucose utilization (R. Levine and M. S. Goldstein, *Rec. Prog. Hormone Res.* **11,** *343 (1954)*; C. R. Park, J. Bornstein and R. L. Post, *Am. J. Physiol.* **182,** *12 (1955)*; E. J. Ross, *Nature (London)* **171,** *125 (1953)*). In order to study penetration phenomena, it is necessary to dissociate the processes of intracellular transport and intracellular utilization. This has been accomplished in a variety of laboratory animals by using several poorly

metabolized pentoses as models for glucose in studies designed to elucidate the mechanisms of sugar transport and insulin action (M. S. Goldstein, W. L. Henry, B. Huddlestun and R. Levine, *Am. J. Physiol.* **173,** *207 (1953)*; E. Helmreich and C. F. Cori, *J. Biol. Chem.* **224,** *663 (1957)*; D. M. Kipnis and C. F. Cori, *Ibid.* **224,** *681 (1957)*). Information concerning pentose metabolism, and the influence of insulin thereon in man, is fragmentary. Recently S. Segal, J. Wyngaarden and J. Foley (*J. Clin. Invest.* **36,** *1383, 1395 (1957)*) reported a group of studies concerned with the metabolic fate, physiologic disposition, and effect of insulin on several pentoses in human subjects.

The rate of pentose disappearance from blood was determined by drawing blood samples at five to ten minute intervals following the intravenous infusion of 10 to 20-gram quantities of pentose. When a curve of falling levels had been established, 0.1 unit of regular insulin per kg. body weight was injected intravenously, and the frequent sampling continued. The removal of these sugars (*i.e.* D and L-arabinose, D-xylose and D-lyxose) followed the kinetics of a first order process. Pentose levels were measured in whole blood, and the rate constants were calculated from the experimentally determined biologic half-time and expressed in terms of per cent pentose disappearing per minute. It has recently been shown by Helmreich and Cori (*loc. cit.*) that when pentoses are determined in whole blood rather than in plasma, their rate of disappearance is underestimated and, in general, values are obtained which are inaccurate for calculating their distribution between blood and tissue. Therefore, the values obtained by Segal, Wyngaarden and Foley are probably on the low side. The rate constants for all the pentoses studied were similar, and ranged from 0.72 to 1.58. Actually, these constants represent the summation of the rate constants of several concurrent processes, namely renal excretion (40 to 60 per cent of the infused pentose was recovered in the urine), utilization, and intracellular penetration. When compared to similarly measured rate constants for glucose, 3.47, fructose, 3.84, and galactose, 6.93, (in the case of these hexoses renal excretion is negligible and the constant approximates that of utilization) the relatively slow rate of pentose metabolism in man becomes readily apparent.

A latent period of ten minutes elapsed between the intravenous injection of insulin and the appearance of a two- to three-fold increase in the rate of disappearance of D-xylose and L-arabinose. This accelerated rate of pentose removal lasted only 20 to 30 minutes (although hypoglycemia persisted much longer) and was followed by an abrupt return to the rate initially observed. Comparison of the blood level found 30 minutes after insulin administration with that obtained by extrapolation of the initial curve revealed differences of 30 to 35 per cent. Since urinary loss was not accelerated by insulin, these results were interpreted as indicating that the hormone accelerated the rate of pentose entry into the peripheral tissues (*e.g.* muscle) thereby increasing its volume of distribution in the total body water. In support of this interpretation, the authors calculated the volumes of D-xylose and L-arabinose distribution, in the presence and absence of administered insulin, and found that they were increased one and one-half times by the hormone. Under similar experimental conditions D-arabinose and D-lyxose did not respond to insulin. Because of the possibility that small changes in the rate of disappearance could occur in the post-insulin period without being detected with the single injection technique, two experiments were performed with D-lyxose and D-arabinose using a constant infusion procedure. Under these conditions D-lyxose showed a minimal response to insulin whereas D-arabinose remained unaffected. To dissociate the pentose response to insulin from the concurrent hypoglycemia, several studies were performed in which glucose was infused immediately after the injection of insulin. Neither the disappear-

ance rate of pentoses nor their responsiveness to insulin was affected by glucose in molar ratios to pentose as high as ten. This suggests that pentoses do not compete with glucose for intracellular penetration.

The conversion of certain pentoses to glucose was suggested by the finding that the infusion of 5 to 20 g. of D-xylose or L-arabinose resulted in a 10 to 30 per cent increase in blood glucose levels. On infusion none of the pentoses affected either the blood lactate or pyruvate concentrations, but all caused a significant decrease in the level of serum inorganic phosphate, this being most marked in the case of D-xylose. In order to obtain information on the fate of the relatively large fraction of D-xylose which did not appear in the urine, two experiments were performed with D-xylose-1-C^{14} in which the disposition of the isotope was determined. Virtually all of the C^{14} recovered from the urine was found in the xylose band on chromatography, less than 0.1 per cent appearing as xylitol or xylonolactone which are two possible metabolites. The expired CO_2 was maximally labeled 45 minutes after the infusion ended, although the first CO_2 sample collected five minutes after D-xylose-1-C^{14} was injected was already appreciably labeled. C^{14} was present in expired CO_2 in detectable amounts for six hours and the total $C^{14}O_2$ expired represented 13.5 per cent of the administered label. The similarity of the CO_2 labeling pattern after C^{14} D-xylose and C^{14} D-glucose suggests that these sugars probably share common intermediary pools which have a rapid turnover. The fate of the residual 30 per cent of the administered label, as well as of the lower four carbons of the xylose whose first carbon appeared as CO_2, remains obscure.

Although the phosphogluconic acid cycle (in which a number of pentose phosphates occur as intermediates) was first demonstrated in plants and bacteria, there has now accumulated abundant evidence to indicate that similar biochemical processes are present in mammalian tissues. The occurrence of pentosurias in humans associated with congenital defects, drug ingestion, and neurological disease suggests that pentoses are of fundamental biological importance. The papers of Segal, Wyngaarden, and Foley represent one of the first attempts to clarify pentose metabolism in man, and, additionally, to offer supportive evidence obtained in the human of the membrane theory of insulin action.

FOOD INTAKE IN THE ARCTIC

It has been assumed that people in very cold regions require considerably more calories than do those living in milder parts of the world. The arctic expeditions again have focused attention on the food requirements of individuals going to polar regions.

Evidence for an inverse relation between the external temperature and the caloric intake was presented by R. E. Johnson and R. M. Kark (*Science* **105,** *378 (1947)*). This relationship was based on dietary surveys of troops stationed in camps at various places in North America. It was suggested by Johnson and Kark that the increased caloric intake in the colder regions was related to the low environmental temperature and to the impedance to work resulting from the cumbersome protective clothing required.

Recently, two reports have appeared which indicate that men in arctic areas consume no more calories than do their counterparts living in temperate areas. One of these presents the dietary data collected during the British North Greenland Expedition (J. P. Masterton, H. E. Lewis, and E. M. Widdowson, *Brit. J. Nutrition* **11,** *346 (1957)*). This expedition was organized as a geological survey of Dronning Louise Land in Northeast Greenland (H. E. Lewis and J. P. Masterton, *Lancet* **269,** *494 (1955)*).

The group disembarked in a fjord on the eastern coast of Greenland in August 1952. All supplies and equipment had to be unloaded by hand. Doing this required great physical effort. Considerable energy was also involved in establishing the base camp 200 miles inland. However, after the base camp was set up, the men spent most of their time in sedentary tasks. The living quarters and laboratory at the base were heated. During the winter, the men spent most of their time indoors.

Two dietary studies were carried out on four of the men 17 months after their arrival in Greenland. These men ranged in age from 23 to 26 years. They were slightly overweight, with the lightest man being 166 cm. (65 inches) tall and weighing 70 kg. (154 pounds) and the heaviest man being 180 cm. (70 inches) tall and weighing 87 kg. (191 pounds).

A typical daily menu available to the men at the base camp consisted of the following.

Breakfast: Porridge or cornflakes, a cooked dish such as kippers or scrambled eggs (made with dried eggs), bread, butter, jam, tea or coffee.

Mid-morning: Tea or coffee, biscuits or bread.

Lunch: Soup, usually Scotch broth, tomato or pea, boiled beef, potatoes and cabbage, stewed fruit with custard, coffee or tea, and bread.

Mid-afternoon: Tea and biscuits, perhaps with rich cake or some baked potato cakes.

Dinner (usually two courses, sometimes three, but always a sweet if lunch had included soup and vice versa): Soup, bacon-and-egg pie, green peas, fried chipped potatoes, steamed pudding, coffee or tea, and bread.

Supper: malted milk, coffee, cocoa, tea, or other beverage. Eggs, kippers, or other snacks could be cooked by anyone who wished.

On two separate occasions food intake was measured. The caloric content was determined by calculation using standard tables of food composition.

During the first survey, which lasted for at least four days, the caloric intake ranged from 3300 to 5090 calories. In spite of the high caloric intake, body weight was either maintained or slightly reduced. It is a problem to reconcile the caloric balance with a graphic presentation of the hours spent at various types of work. Less than one hour of heavy work per day was put in by the men during this time; one man who had the lowest caloric intake put in four hours of medium work. The rest of the time was spent sitting (four to thirteen hours) or sleeping (six to eight hours).

According to Masterton, Lewis and Widdowson (*loc. cit.*), the caloric intake and expenditure of the men at the base camp in Greenland was similar to those of cadets at a military establishment in England.

It should be pointed out that the men at the base camp spent an average of 11½ hours sitting each day while the cadets spent only seven hours so occupied (O. G. Edholm, *et al.*, *Brit. J. Nutrition.* **9,** *286 (1955)*). The men at the base camp spent an average of four and one-half hours a day at physical work "but very little of it could really be called heavy." The cadets expended approximately 17 per cent of their total energy output on walking and cycling and another 15 per cent on sports and drill.

The daily intake of the 12 cadets averaged 3432 calories (Edholm, *et al. loc. cit.*) whereas that for the four men at the base camp averaged 3900 calories (Masterton, Lewis and Widdowson, *loc. cit.*). Although one can accept the difference of 470 calories as the experimental error of the technique, the difference is probably greater than this since the men at the base camp in Greenland were probably less active than the cadets.

The above four subjects paired off for sledging expeditions in the spring. Two of the men were gone for ten days, and the other two went for 19 days. The food consumed on the expedition was computed from the difference in the weight of food taken

from, and that returned to, the base camp. Plate waste was reported to be absent and, for this reason, the investigators felt they had an accurate measure of the average daily food consumption. No indication is given as to how the caloric intake was apportioned between the two men on the sledging expeditions. The daily caloric intake ranged from 4520 to 4870 calories during the sledging expedition. The hard work entailed during the expedition resulted in a slight loss of weight by one subject but no change in weight for the other three. The food provided the men during the sledging expedition consisted of (in ounces per man per day): pemmican, 5.6; butter, 5.6; potato powder, 1.6; cocoa, 0.4; chocolate, 2.4; sugar, 3.0; oats, 2.3; dried milk, 1.6; biscuits (high-fat), 4.3. This ration provided 4164 calories daily, and contained 99 g. protein, 278 g. fat and 310 g. carbohydrate. The difference between the caloric value of the latter ration and that reported as being consumed by the men was presumably made up by extra chocolate, raisins, milk, and biscuits.

One of the reasons for carrying out the dietary survey was to determine whether men in the Arctic lost large amounts of fat in their stools. That this was a possibility was suggested by the observation that the dogs used on Arctic expeditions avidly consumed human feces while dogs in other parts of the world were not so disposed. To answer this problem, stool samples were collected from the men on the sledging expedition. These men excreted an average of 163 g. of feces a day, which, by analysis, contained a normal percentage of fat and nitrogen. The weight of the feces was approximately one and one-half times those usually considered normal. The analytical results offered no explanation for the peculiar appetite of the dogs.

The second paper is by J. A. LeBlanc of the Defence Research Northern Laboratory at Fort Churchill, Canada (*J. Applied Physiology* **10,** *281 (1957)*). He compared the reported food intake of soldiers undergoing tests at various northern stations (most of them at Fort Churchill). Except for one group at Fort Churchill, the food intake per man per day ranged from 3400 to 4000 calories. These values are very similar to those reported for soldiers in temperate climates.

Both of the above reports emphasize the fact that protective clothing and the ability to heat the base camps have reduced the actual caloric requirement of individuals working or living in the arctic. However, as LeBlanc (*loc. cit.*) points out, many individuals are likely to consume excessive amounts of food due to the environmental isolation, the length of the day, and the crowded living conditions. He states that the average weight gain of 40 employees in the laboratory was about seven pounds over a period of one year. The latter factors, and not the cold *per se*, probably account for most of the reported increased caloric intake of individuals in arctic regions.

SERUM GLUTAMIC-OXALACETIC TRANSAMINASE ACTIVITY FOLLOWING OPERATION

Large increases in the glutamic-oxalacetic transaminase activity observed in the blood serum of patients having a recent myocardial infarction have been used as a diagnostic sign in this disorder. Smaller increases have been noted in individuals with liver necrosis or muscle injury. A slight increase may occur, however, in the serum following surgical procedures. Recently, W. L. Craver, G. Johnson, Jr., and J. M. Beal (*Surg. Forum* **8,** *77 (1958)*) have reported a study which was undertaken to define the magnitude and duration of this rise in enzyme activity.

Transaminase activity was measured in

the serum of 20 patients operated upon for various reasons. Two pre-operative venous blood samples were drawn for transaminase determination, one having been obtained immediately before the induction of anesthesia. After operation blood samples were obtained at two-hour intervals for the first six to ten hours. During the next five to eight days a blood sample was drawn every 24 hours until the transaminase activity returned to the control level. Since the serum transaminase activity increases greatly following a myocardial infarction, electrocardiograms were obtained before and after operation in most of the patients and no evidence of myocardial infarction was found.

As indices of liver function serum alkaline phosphatase, thymol turbidity, bilirubin and the bromsulphalein excretion test were evaluated in the majority of the patients. No significant alterations were detected. It was found that the maximum rise in serum transaminase activity occurred within two to four hours postoperatively and then underwent a rapid decline, although the control levels often were not reached for several days following the operation. Normal non-operated individuals were found to have serum transaminase activity ranging between 8 and 40 units per ml. per minute.

One patient whom these investigators studied following a partial pancreatectomy, partial gastrectomy, left adrenalectomy and splenectomy, showed a serum transaminase activity of 200 units per ml. per minute two hours postoperatively. In spite of this high value, the normal level was reached by the fourth post-operative day. Two other patients, one undergoing an esophageal resection and the other a total gastrectomy, had serum transaminase levels of 158 and 137 three or four hours postoperatively. In another series consisting of 13 surgical patients who had partial gastric resection, repair of a hiatus hernia, cholecystectomy, sigmoid resection, or cystoscopy, the maximum transaminase levels never exceeded 70 units per ml. per minute. Also it was noted that neither general anesthesia nor minor urological procedures induced significant increases in transaminase activity.

Large increases in serum transaminase activity have been detected in patients undergoing extensive surgical procedure. Since there is a sharp rise in the level of enzyme activity followed by a steep decline in activity, and because there is no evidence of cardiac or liver damage, it is believed that the postoperative increase in serum transaminase activity is due to necrosis of skeletal muscle and other tissue induced by the surgical trauma.

INCREASED RENAL CALCIUM CLEARANCE WITH VITAMIN D

The actions of vitamin D in the body are multiple and are reflected in diverse organs. It is generally accepted that vitamin D promotes absorption of calcium from the gastrointestinal tract. An excessive absorption of calcium from the gut accounts for the hypercalcemia of hypervitaminosis D. A somewhat similar situation has been described in sarcoidosis by P. H. Henneman, *et al.* (*J. Clin. Invest.* **35,** *1229* (*1956*)) and in infantile hypercalcemia (*Nutrition Reviews* **14,** *338* (*1956*)).

Vitamin D also has a direct action on bone. An increase in the rate of accretion and resorption of bone minerals has been demonstrated by isotopic methods (*Nutrition Reviews* **15,** *70* (*1957*)) and alterations in bone citrate follow the administration of vitamin D to deficient animals (*Nutrition Reviews* **16,** *140* (*1958*)).

A third action of vitamin D is upon the renal handling of phosphorus, although the literature on this subject is most confusing. Vitamin D has been variously reported to

increase or decrease the renal clearance of phosphate. When given to vitamin D deficient animals the excretion of phosphate and its renal clearance both decrease. J. D. Crawford, D. Girbetz and N. B. Talbot (*Am. J. Physiol.* **180,** *156 (1955)*) have discussed the difficulty in interpreting such observations in animals possessing intact parathyroid glands. When parathyroidectomized rats were studied these authors found that vitamin D increased the clearance of phosphate. A comparable effect is observed clinically when vitamin or dihydrotachysterol are used to treat patients with hypoparathyroidism. Under these circumstances the high serum phosphate characteristic of this disease decreases toward normal. In vitamin D resistant rickets the phosphaturic effect of vitamin D may be a hindrance in preventing successful treatment of the rickets (*Nutrition Reviews* **14,** *176 (1956)*).

The possibility that vitamin D might affect calcium resorption by the renal tubule has aroused recent interest. A number of clinical observers have found increased calcium excretion in patients with hypoparathyroidism under treatment with vitamin D. This abnormality has been studied specifically in patients in the clinic of Fuller Albright by J. Litvak and co-workers (*J. Clin. Endocrinol.* **18,** *246 (1958)*).

Simultaneous measurements of the 24-hour urinary calcium excretion and serum calcium levels were available in 21 patients with either spontaneous hypoparathyroidism, hypoparathyroidism following thyroid surgery or pseudohypoparathyroidism (a condition in which the renal tubules are apparently refractory to parathyroid hormone). Urinary calcium excretions were low in patients before treatment. When either vitamin D_2 in oil or dihydrotachysterol were given, calciuria in excess of 150 mg. per day occurred in two thirds of the patients with true hypoparathyroidism but not in any of the patients with pseudohypoparathyroidism.

It is particularly noteworthy that five patients exhibited hypercalciuria at a time when hypocalcemia was present. Over a gram of calcium was excreted by one patient daily with a serum calcium level of only 4.6 mg. per 100 ml. It is true, however, that this patient had a renal infection at the time of study. There was no relation noted between calciuria and the amount and type of calcium supplements used in therapy. The fact that hypercalciuria often occurred at a time when serum phosphate values were still elevated suggests the independence of the calciuric and phosphaturic actions of vitamin D. Two of the ten patients with hypercalciuria developed calcium oxalate stones.

No direct evidence was offered concerning the mechanism of hypercalciuria in these patients although strong arguments are presented that a decrease in tubular reabsorption of calcium was responsible. The amount of calcium in serum available for filtration at the glomerulus is not increased by vitamin D (T. R. Hopkins, T. B. Connor and J. E. Howard, *Bull. Johns Hopkins Hosp.* **93,** *249 (1953)*). Only a few investigators have tackled the difficult technical problems of performing accurate renal clearance studies of calcium, and much remains to be learned concerning the effect of vitamin D and other agents.

In addition to theoretical interest, the hypercalciuria associated with high doses of vitamin D and related sterols is of practical importance to physicians treating patients with hypoparathyroidism. Estimating the calcium excretion with the Sulkowitch test has been a guide to therapy in the past. It is now apparent that normal or increased urinary calcium levels may occur with *hypo*calcemia. In certain patients the physician must guide a course between hypocalcemia and massive hypercalciuria. It is doubtful whether the smaller doses of vitamin D used in the treatment of rickets exert any specific effect on calcium reabsorption by the renal tubule.

THE USE OF VOLATILE FATTY ACIDS BY RUMINANTS

Studies of the mechanism by which ruminant animals are able to make use of rough fodders as their main source of energy have shown that much of the cellulose and other carbohydrates in these feeds is fermented by rumen bacteria, with volatile fatty acids as the main end-products. The efficiency of utilization of rough fodders must therefore depend in part on how efficiently the metabolizable energy of acetic, propionic acid and butyric acid can be harnessed to the needs of the animal. G. L. McClymont (*Australian J. Sci. Res. B*, **5**, *374 (1952)*) reviewed the data pertinent to this question and concluded that in view of the known metabolic pathways for acetic acid the energy available in this compound was unlikely to be used efficiently, and that the generally poorer utilization of metabolizable energy by ruminant animals, as compared to non-ruminant animals, might be due mainly to this factor rather than to losses of free energy in microbial fermentation or to the earliest explanation of the physical work required to push the roughage through the alimentary canal.

A major investigation of the utilization of the various volatile fatty acids singly and in combination has been undertaken at the Hannah Dairy Research Institute in Scotland. In their first paper, D. G. Armstrong and K. L. Blaxter (*Brit. J. Nutrition* **11**, *247 (1957)*) describe their method of experimentation. Adult sheep fitted with a fistula into the rumen were housed in pens, and transferred as required to a respiration chamber for indirect calorimetry. The design of the chamber and the analytical methods used have been described by Blaxter, N. McC. Graham and J. A. F. Rook (*J. Agr. Sci.* **45**, *10 (1954)*).

The sheep were first studied for two days while being fasted, and then were given continuous drip infusion of a fatty acid solution with an equal volume of physiological "saline" solution for a further 48-hour period. At the end of this period observations were continued during a second two-day period of fasting. When each acid was administered separately, at the rate of 700 calories per 24 hours, the heat increment as a percentage of the metabolizable energy given was 41 per cent for acetic acid, 14 per cent for propionic acid and 16 per cent for N-butyric acid. The high value obtained with acetic acid was confirmed in two further trials, and also checked by measurement of carbon and nitrogen balance; this again agreed with the usual calculations based on oxygen consumption and respiratory quotient with the necessary corrections.

Even at the starvation level, therefore, where animals can usually make more efficient use of energy sources than at higher planes of nutrition, the acetic acid given was used with only 59 per cent efficiency, as compared with an efficiency of 94 per cent for glucose given in a parallel trial under conditions of minimal fermentation (Armstrong and Blaxter, *Proc. Nutr. Soc.* **15**, *1 (1956)*).

Various measurements were taken to check as far as possible whether or not the conditions under which the acids were given remained "physiological" rather than "pharmacological." The concentration of volatile fatty acids in the rumen contents was 7.7 mEq per 100 ml. after 24 hours of dosage with acetic acid, and was still at this level after 48 hours, which indicated an equilibrium state. With the other acids the concentration, in terms of milliequivalents, was lower and in proportion to the size of the dose (*i.e.* the same number of calories was provided by fewer of the large molecules). None of the values was outside the range of figures that have been obtained for the rumen liquor of sheep receiving normal rations.

At the beginning of the infusion period

the blood sugar level was approximately 34 mg. per 100 ml. After 48 hours of infusion with propionic acid this had risen to 48 mg. per 100 ml. and there was no increase in the level of steam-volatile fatty acids in the blood; but with acetic acid and N-butyric acid there was in each case an approximate halving of the blood sugar level and a significant rise in the level of volatile fatty acids. With butyric acid there was an increase in the level of circulating ketone bodies (expressed as acetone) from an initial level of 4 mg. to 33 mg. per 100 ml. With acetic acid there was a small increase, and with propionic acid there was none. The contrast between the response to butyric and propionic acids is in line with the findings of metabolic studies with rumen epithelium tissue reported by R. J. Pennington (*Biochem. J.* **51,** *251 (1952)*) and Pennington and T. M. Sutherland (*Biochem. J.* **58,** *vii (1954)*).

The unusually high concentration of volatile fatty acids (1.0 mEq per 100 ml.) remaining in the blood stream after administration of acetic acid suggests that the tissues of the sheep are unable to metabolize this compound rapidly, although it is believed to be a major end-product of rumen fermentation. However, in practical conditions it is present together with the other volatile fatty acids which may have a synergistic effect on the utilization of acetic acid. Further experiments reported by Armstrong, Blaxter and Graham (*Brit. J. Nutrition* **11,** *392 (1957)*) provide strong evidence for the existence of such an effect.

In the second series of experiments a longer period of infusion, either 72 or 96 hours, was generally used and the first 24 hours were regarded as a period of adaptation before respiratory measurements were commenced. Under these conditions a supply of 700 calories per 24 hours from acetic acid again resulted in 41 per cent of the energy appearing as heat increment. However when 700 calories were supplied in a mixture with the molar ratio of acetic acid 90, propionic acid 6 and n-butyric acid 4 (so that 38 per cent should have appeared as increment if there were no interaction effects, and calculating from the results with the acids given singly), only 15 per cent of the calories appeared as the observed heat increment. In fact the mixture was utilized almost as efficiently as if it were entirely composed of one of the higher acids. Moreover the mixture apparently spared the loss of urinary nitrogen, whereas giving acetic acid alone had resulted in an increased protein breakdown.

The same profound effect on the utilization of acetic acid was observed with the administration of mixtures in different proportions. Mixtures in which the molar proportion of acetic acid ranged from 25 to 90 per cent had heat increments varying only from 13 to 17 per cent. Since the observations made on the composition of the volatile fatty acid mixture in the rumen liquor of sheep fed natural rations have shown the proportion of acetic acid always to be well within these limits, the authors conclude that this factor is unlikely to explain the wide variations observed in the utilization of metabolizable energy by sheep fed on different rations at a sub-maintenance level.

It is suggested that the synergistic effect is related to the entry of two-carbon and four-carbon components into the tri-carboxylic acid cycle; and that when acetic acid is given alone to a fasting sheep it causes a drain on glucose circulating in the blood stream, and so stimulates gluconeogenesis from protein with an increased loss of urinary nitrogen. Apparently, however, even the addition of one molecule of propionic acid for every fifteen molecules of acetic acid metabolized is a sufficient source of carbohydrate-forming material to prime the cycle of carbohydrate metabolism, and thus to utilize the acetic acid efficiently and reduce the breakdown of body protein.

Under fattening conditions it appears that more may depend on the nature of the volatile fatty acids produced. In their work

with sheep fed at above the maintenance level Armstrong and Blaxter (*Brit. J. Nutrition* **11,** *413 (1957)*) find acetic acid to be inferior in its utilization to propionic and butyric acid even when added as a low level supplement to the main ration of dried grass, which should have provided adequate metabolites for the type of synergistic effect observed under maintenance conditions.

NUTRITION AND THE ONSET OF REPRODUCTIVE ACTIVITY

Undernutrition leads to symptoms that have been described as a condition of "pseudohypophysectomy", because they are so similar to the changes seen after removal of the pituitary (*c.f.* review by L. T. Samuels, *Recent Progress in Hormone Research* **1,** *147 (1945)*). Furthermore some of the symptoms of undernutrition, such as regression of the reproductive organs can be reversed by the administration of pituitary hormones.

Most of this work has been done with rats and mice, and the activity of the reproductive organs has been gauged by sacrifice of the animals at successive intervals. D. V. Davies, T. Mann and L. E. A. Rowson (*Proc. Roy. Soc. B,* **147,** *332 (1957)*) have now made a study of the onset of male sex hormone activity and sperm formation in calves reared on varying levels of feed intake. For this work methods were developed to permit a continuous study of the development without sacrifice of the animals; similar procedures may prove valuable in other studies with large and nonexpendable species.

In each of three successive trials a pair of "identical twin", monozygous bull calves was used to reduce "non-treatment" variability. From approximately two months of age in each case one twin was given a very liberal diet of good quality, and the other less than half of the quantities eaten by the "high-plane" animal. Attempts were made to obtain semen from the calves every one or two weeks by means of an electrical stimulus as described by L. E. A. Rowson and M. I. Murdoch (*Veterin. Rec.* **66,** *326 (1954)*), but with a modified electrode for insertion into the rectum.

The first trial was continued until the calves were five and one-half months old, when the animals weighed 146 and 102 kilograms, respectively. The latter, on the "low-plane" of nutrition had received an average daily ration of 0.5 kg. of concentrates (oats 30, barley 20, bran 25, linseed 10, bean meal 10, fish meal 5), 1 kg. of roots, 2 kg. of hay, and 15 g. of cod liver oil. This animal failed to give any ejaculates, while these were obtained regularly from the "high-plane" calf for the last six weeks of the trial, though they contained no spermatozoa.

The calves were then slaughtered. The testes, epididymides and seminal vesicles were all nearly twice as heavy in the "high-plane" calf. Chemical analysis showed even greater differences in the concentrations of fructose and citric acid in the seminal vesicles of the two animals. In the under-fed animal they contained approximately 35 mg. of fructose and 50 mg. of citric acid per 100 ml., while those of the other calf contained three times the concentration of both substances. Histological examination showed, as expected, a greater development of functional tissue in the reproductive glands of the better fed animals.

The second trial was of similar design, but continued until the calves were 10½ months old. Their weight difference gradually increased until they finished at 380 and 285 kg., respectively. It was found possible to obtain semen samples from the under-fed calf from five months of age onwards, and so to compare the composition of the samples from the two animals. The volume of each ejaculate from the well-fed calf increased gradually to approximately 10 ml. at nine

months of age, while the corresponding volume from its twin was usually one-third, or less.

The samples obtained showed striking contrasts in their composition. Up to nine months of age the "low-plane" semen contained no more than 10 mg. (per 100 ml.) of either fructose or citric acid; the concentrations in the "high-plane" semen increased rapidly during the sixth month to approximately 70 mg. (per 100 ml.) of each substance. A similar rise in the semen from the under-fed animal occurred only in the tenth month. The concentrations of these substances, in fact, differentiated the two animals more sharply than did counts of spermatozoa. Spermatozoa first appeared in the "high-plane" semen at nine months of age, and in the semen from the under-fed calf only three weeks later.

These animals were again sacrificed at the end of the trial, and the reproductive organs were examined. They were considerably heavier in the "high-plane" than in the "low-plane" animal, but there was less difference in the histological picture than was seen in the younger twins of the first trial. Differences were, however, still present, particularly in the development of peripheral cells in Cowper's gland.

The final trial followed the general pattern of the first two. In addition, the calves received a series of injections from four and one-half months of age, after semen samples had started to be obtained from each. In all, eight injections were given, over 15 days, each containing 2,000 I.U. of purified gonadotrophin prepared from human pregnancy urine. There was an immediate response in the output of citric acid and fructose in the semen from each calf, but the quantities were much greater in the "high-plane" animal, the highest concentrations being over 300 mg./100 ml. of each. The corresponding figures for the "low-plane" calf were between 60 and 70 mg./100 ml. It seems certain that this was a response to the gonadotrophin, since within a week of their ending even the "high-plane" ejaculates contained less than 30 mg./100 ml. of either fructose or citric acid.

The semen from the well-fed animal later showed the normal rise in fructose and citric acid levels at six and one-half months of age, whereas in the under-fed twin it only began at eight months of age. By 13 months of age, when bulls are considered sexually mature, the "low-plane" animal was secreting nearly as much fructose, citric acid and total volume of semen as its control bull, but still in the last month gave only 1.6 x 10^9 sperms while the "high-plane" animal gave three times as many.

At autopsy the animals weighed 385 and 245 kg., respectively, and the weights of the testes and seminal vesicles were in proportion. However, there was much less difference in weight for the epididymides and ampullae. Differences in histological appearance had again been reduced, as the animals were still older at slaughter, but they remained distinguishable. The under-fed animal showed less lipid in the epithelial cells of the seminal vesicles, and the epithelial cells in Cowper's gland were not of the fully adult type found in the "high-plane" twin.

These experiments therefore confirm the effect of feeding on the development of sexual maturity in bulls; and the results suggest that, as with laboratory animals, delayed development with under feeding is associated with a reduced pituitary activity. It is also evident that obtaining ejaculates of semen, by electrical stimulation, and analysing these for their content of fructose or citric acid can be used as a measure of sexual development for a considerable period before the appearance of spermatozoa. Such a simple procedure may therefore be used to provide useful additional information during the progress of large-animal feeding trials, and so contribute to our general knowledge of the relative effect of deficiencies of different nutrients on endocrine function.

EXPERIMENTAL XANTHOMATOSIS

The feeding of cholesterol to rabbits for prolonged periods results in the deposition of cholesterol in the skin, joints, and bones, and produces lesions closely resembling hypercholesterolemic xanthomatosis in man. Several aspects of this disease have been reviewed recently (*Nutrition Reviews* **11,** *72, 231, 264 (1953)*). The term xanthoma, meaning yellow tumor, is applied to the deposits seen in the tissues in this disease. These nodules are characterized microscopically by having light cells with a foamy cytoplasm.

C. I. Wang, L. Strauss and D. Adlersberg (*Arch. Path.* **63,** *416 (1957)*) reported their systematic study of the characteristics of experimental xanthomatosis in the rabbit including the incidence, clinical and roentgenological manifestations, blood lipid changes and gross and microscopic changes in the skin, joints and bones. The animals used in this experiment consisted of a group of 120 male rabbits weighing between 2.5 and 3.0 kg. which received about 1 g. of cholesterol daily from their diet of cholesterol-treated chow. The feeding intervals ranged from two weeks to one year. Blood samples taken every two to four weeks were used to determine the amounts of circulating cholesterol, phospholipids and total lipids.

In order to obtain information about the early changes occurring in the development of atherosclerosis and of xanthomatosis in the skin and joints, most of the animals were killed within the first three months of cholesterol feeding. Five of the remaining 33 animals were killed at the end of each subsequent month. The incidence of xanthomatosis increased with the duration of cholesterol feeding. No xanthomata were seen during the first month of cholesterol feeding. After two to three months about 2.5 per cent showed cutaneous xanthomata, as did 21 per cent after three to six months and 50 per cent after seven to nine months; after ten to 12 months 100 per cent of the animals showed skin xanthomata. The plasma lipids increased to a maximum during the first three months then dropped after four to six months and tended to level off after seven to ten months. Although individuals varied greatly, the general trend was similar in all cases. Free plasma cholesterol rose from 50 to 1280 mg. per 100 ml. after one to three months, decreasing to approximately 800 mg. over the period from seven to 12 months. Esterified cholesterol did not change. Plasma phospholipid rose to a maximum of 450 mg. per 100 ml. after four to six months, but remained at a lower level for the remaining period. Total lipid was 6 to 7.5 times normal, becoming highest after three months of cholesterol feeding.

The development of xanthomata in the skin required hypercholesteremia, but there was no correlation between the degree of elevation of blood cholesterol and the time of appearance of the skin xanthomatosis. The duration of cholesterol feeding was an influential factor. The variation in lipid deposition between individuals could have been due to local tissue factors varying from animal to animal. The xanthomata appeared as reddish-yellow nodules from 1 to 3 mm. in diameter between the toes and over the heels. The nodules increased to 1 to 1.5 cm. as the feeding continued and the skin of the soles became thickened and cracked. In animals fed cholesterol for a period of three to six months, small elevated patches 1 to 3 mm. in diameter appeared scattered over the ears. These patches were similar in appearance to eruptive xanthomatous lesions in man. Previous injury predisposed the site to the development of nodules. The skin over the back and neck became reddish and thickened and showed loss of hair in some instances. Ulceration of many xanthomata occurred and secondary infection developed with abscess formation. Sterile abscesses were seen occasionally containing cholesterol-rich white material

which showed no bacterial growth. Swelling and thickening of the ankles and knee joints was found in some of the animals fed cholesterol for more than six months but there was no other evidence of acute inflammatory change.

Serial X-ray studies of animals fed cholesterol from two to eight months indicated extensive thinning of the cortex of the long bones after three to five months. Some of the animals exhibited pathological fractures.

No gross atheromatous plaques were seen until the end of one month of feeding. Tiny, but definite, white plaques could be seen in the ascending part and the arch of the aorta especially around the orifices of the branching arteries. As cholesterol feeding continued, the plaques became larger, more numerous and confluent. Plaques appeared at the orifices of the intercostal arteries and the branching arteries of the abdominal aorta. The mitral and aortic valves became thickened and beaded. Continuation of cholesterol feeding beyond four to six months resulted in such extensive plaque formation in the aorta and pulmonary artery that normal intima could be seen in only occasional small areas.

The earliest microscopically visible xanthomata contained foam cells either isolated or in nests in the upper corium and in perivascular and perineural spaces in the deep corium. The ground substance stained with hematoxylin-eosin showed a bluish-violet hue when foam cells were present. The xanthomatous skin nodule, when examined histologically, showed thinning of the epidermis, distention of the dermis with fat laden foam cells, and a central pool made up of cholesterol crystals and cellular debris. Heavy strands of collagenous fibers of the dermis separated the nests of foam cells.

Roentgenological changes in the bones and joints were preceded by deposition of foam cells in the synovial membrane, peritoneum and bone marrow. Foam cells were also observed in the fibrous sheath of tendons and ligaments. Tissues characterized by having highly differentiated cells and poor blood supply, such as cartilages, tendons and ligaments, showed no foam cells. Intact bones showed fewer foam cells than did bones with fractures.

Changes in the ground substance accompanying experimental xanthomatosis in the rabbit were studied by Wang, Strauss and Adlersberg (*Arch. Path.* **64,** *501 (1957)*). The original observation of a bluish violet hue of the ground substance in sections stained with hematoxylin-eosin led to further studies of the relationship between "acid mucopolysaccharides" of the ground substance as demonstrated by staining with colloidal iron, and the deposition of lipids during cholesterol feeding of rabbits. A group of 120 "chinchilla gray" male rabbits was fed cholesterol-treated chow from two weeks to ten months, receiving approximately 1 g. of cholesterol per day. Of this group, 52 were selected for the study of experimental atherosclerosis and lipoidosis after varying periods of cholesterol feeding. Tissues were fixed in 10 per cent formalin and sections were stained with hematoxylin-eosin.

Although there is some question about the specificity of the procedure, colloidal iron staining was interpreted to indicate the acid mucopolysaccharides of the ground substance and gave a bright turquoise color. For reference umbilical cord was stained by the same technique. The skin of untreated control rabbits showed no foam cells and only small quantities of the colloidal iron positive material in the ground substance of the connective tissue surrounding the hair follicles of the dermis. Animals fed cholesterol showed the early appearance of the blue material coinciding with the appearance of foam cells followed by a decrease in "mucopolysaccharides" as foam cells increased. Lipids seemed to be deposited initially in a fine matrix of this material, both within and outside the cells.

The cartilaginous tissue matrix of the joints of untreated young animals was

characterized by a bright blue color with the colloidal iron stain. Cholesterol feeding was accompanied by the usual deposition of foam cells in the synovial membrane, periosteum, bone marrow and lacunae. A moderate increase in "mucopolysaccharides" was seen in the connective tissue around the arteries, in the periosteum and in the bone marrow along the trabeculae. The cytoplasm of the foam cells was stained pale blue. The difference in staining observed in different normal tissues either indicated a quantitative variation in "acid-mucopolysaccharide" concentration, or reflected a changing color reaction due to the fact that the type of chondroitin sulfate varied from tissue to tissue. For example, chondroitin sulfate B has been found in the ground substance of dermis and chondroitin C occurred in tendon and ligament.

The feeding of cholesterol produced a rapid accumulation of colloidal iron positive material in the skin which normally contained only small amounts. In tissues which normally have large amounts of acid mucopolysaccharides, such as hyaline cartilage of long bones, tendons, and ligaments, neither increase in "acid mucopolysaccharides" nor accumulation of foam cells occurred. In skin the receding of the blue stainable material accompanying increased lipid deposition suggested the possibility that the presence of lipid molecules in tissues could have affected the staining ability by the colloidal iron technique. Correlation of the results of chemical analyses with recession of colloidal iron staining during prolonged cholesterol feeding might be useful in the resolution of this problem.

HISTIDINE AND EXPERIMENTAL ATHEROSCLEROSIS

A report from Russia mentions briefly the various substances that have been used in that country for the treatment of atherosclerosis, and describes an experiment with rabbits which suggests that histidine may be one more substance that will lower the blood cholesterol level.

The author of the above report, G. N. Chimakadze from the Institute of Clinical and Experimental Cardiology of the Academy of Sciences of the Georgian Soviet Socialist Republic (*Farmakologiia i Toksikologiia* **17,** *11 (1954)*), indicates that iodine preparations were widely used in the treatment of atherosclerosis in Russia during the last century. More recently, potassium iodide was reported to lower the blood cholesterol level in atherosclerosis. Sodium fluoride and potassium thiocyanate produced similar results. Ascorbic acid, presumably, lowers the blood cholesterol level of patients with hypertensive disease, and prevents the development of atherosclerosis in experimental animals. Vitamin D_2, on the other hand, favors the development of atherosclerosis. Choline chloride is supposed to delay the development of atherosclerosis produced by cholesterol feeding. Thyroxin has been reported to reduce the incidence of atherosclerosis.

Chimakadze (*Loc. cit.*) was led to study the influence of histidine on atherosclerosis as a result of the recognized property of histidine in suppressing adrenergic innervation, and his preliminary observation that rabbits with high levels of cholesterol show a reduced level of sympathin (an assumed hormone excreted by smooth muscle which causes an increased heart rate) in blood.

In his experimental work, Chimakadze (*Loc. cit.*) fed 20 rabbits cholesterol (0.2 g. per kg.) dissolved in peach kernel oil. After being maintained on this regimen for 45 days, one-half of the animals had their intake supplemented with 80 mg. of histidine per day (optical isomer not stated). The experiment was continued for an additional 20 days.

In the rabbits on the basal ration, the blood cholesterol level increased progressively until it reached a value of 3846 mg. per cent by the sixty-fifth day. The blood pressure, which was determined in a carotid skin loop, increased only slightly. These rabbits showed an apparent change in the response of the cardiovascular system to the topical application of adrenalin (applied to the buccal mucosa). As the experiment progressed, the pressor effect of adrenalin increased until, by the sixty-fifth day, it amounted to 25 to 30 mm. of pressure, which was maintained for 45 to 60 minutes. It was stated that five control rabbits, which received only the peach kernel oil added to the diet, showed no change in the reactivity of the circulatory system. Morphological examination of the blood vessels indicated an extensive atherosclerosis in those animals receiving only the cholesterol.

The rabbits receiving the histidine in addition to the cholesterol showed a marked reduction in the blood cholesterol level. This was apparent by the tenth day of the histidine therapy, when the level had been reduced by 600 to 800 mg. per cent. On the twentieth day, the level had dropped by 1000 to 1300 mg. per cent. The reactivity of the circulatory system to adrenalin was also restored to normal following the histidine administration. Morphological examination of the blood vessels indicated a very mild degree of lipid infiltration of the intima. Atherosclerotic plaques were seen in the abdominal aorta in only one of the rabbits receiving histidine.

The above observations are interesting in suggesting that a relatively small amount of histidine has a very pronounced effect on the blood cholesterol level of rabbits. If a similar phenomenon occurred in man, it would mean that 2.8 g. of histidine per day should be effective in a 70 kg. man. Confirmation of Chimakadze's findings (*Loc. cit.*) will be eagerly awaited and, if they are confirmed, trials with human beings would be of interest.

PHYSIOLOGICAL ROLES OF CARNITINE

Since the discovery of carnitine as a necessary growth factor for several insect species, evidence has accumulated regarding its universal occurrence in biological materials (G. Fraenkel, *Arch. Biochem. Biophys.* **50,** *486 (1954)*). It has been postulated that carnitine has an important physiological function in all organisms. The majority apparently obtain it by biosynthesis, while a few must have it supplied as a "vitamin" in the food.

Measurable quantities of this substance were found to be synthesized during the development of the chick embryo (Fraenkel, *Biol. Bull.* **104,** *359 (1953)*). Thus, when subsequently it was discovered that γ-butyrobetaine (desoxycarnitine) acted as a specific inhibitor for carnitine in the development of the mealworm *Tenebrio molitor* (P. K. Bhattacharyya, S. Friedman, and Fraenkel, *Arch. Biochem. Biophys.* **54,** *424 (1955)*), the opportunity was provided to demonstrate the physiological importance of carnitine in an organism which normally synthesizes the substance (T. Ito and Fraenkel, *J. Gen. Physiol.* **41,** *279 (1957)*).

Cultivation of chick blastoderms was performed on synthetic media, following the technique developed by N. T. Spratt, Jr., (*J. Exp. Zool.* **110,** *273 (1949)*). The composition of the medium was as follows: Chick-Ringer solution; Agar to a concentration of 0.6 per cent; 1.25×10^{-3} M glucose. Sterile solutions of γ-butyrobetaine and carnitine were added in varying amounts. Microscopic observations of the embryos were made after 20, 40 and 60 hours. Developmental stages of the blastoderms were examined and

described according to the terminology of V. Hamburger and H. C. Hamilton (*J. Morph. Physiol.* **88,** *49 (1951)*).

Explanted blastoderms of definite streak or head-process stage were found to develop in the presence of glucose. Formation of a brain, spinal cord, optical vesicle, somites and heart were ordinarily observed and in most cases pulsations of the heart could be detected.

With a relatively high concentration of γ-butyrobetaine (5×10^{-2}M or 3×10^{-2} M) almost all the explants, after a slight initial development during the first 20 hours, degenerated during the next 20 hours. On media containing 2×10^{-2} M butyrobetaine more than half of the embryos succeeded in approaching normal development and differentiation, while the remainder developed for 20 hours and then underwent degeneration. With 1×10^{-2} M butyrobetaine practically all the embryos developed at least to the stage just preceding heart pulsation, while 5×10^{-3} M had no adverse effect.

With 5×10^{-2} M butyrobetaine in the medium, the addition of 5×10^{-3} M L-carnitine had a slightly detrimental effect, but addition of 2×10^{-3} M L-carnitine improved development slightly. In the case of 3×10^{-2} or 2×10^{-2} M butyrobetaine, the addition of 2×10^{-3} or 1×10^{-3} M L-carnitine definitely counteracted the inhibition of development induced by γ-butyrobetaine. Eleven of 30 explants degenerated with addition of 2×10^{-2} M butyrobetaine and in none of 45 did this occur after treatment with 2×10^{-3} or 1×10^{-3} M L-carnitine. However, with lower concentrations of butyrobetaine, addition of carnitine had no effect.

Since γ-butyrobetaine inhibited development and differentiation of the early blastoderms, experiments were carried out to determine if some type of inhibition could be induced in older blastoderms. Most explants of the 1-somite through the 4-somite stage developed normally at a concentration of γ-butyrobetaine as high as 5×10^{-2} M, and none degenerated without some signs of development. A concentration of 2×10^{-2} M or lower had no effect. The addition of L-carnitine in concentrations of 2×10^{-3} or 1×10^{-3} M had some favorable effect on the development of blastoderms placed on media containing 2×10^{-2}, 3×10^{-2} or 5×10^{-2} M γ-butyrobetaine. In a few explants the heart beat was restored as an effect of added carnitine.

DL-Carnitine in concentrations of 1×10^{-2}, 4×10^{-2} or 1×10^{-1} M had increasing inhibitory or toxic effects on the early blastoderms. The symptoms of degeneration were the same as in the presence of similar concentrations of γ-butyrobetaine. The authors are of the opinion that the inhibitory effect was not due to the use of DL carnitine instead of L-carnitine. In feeding experiments with the mealworm, *Tenebrio*, DL-carnitine had one-half the activity of L-carnitine and the D component, while physiologically inert, proved harmless.

These studies point up the critical dependence on concentration in experiments of this type. There was a relatively narrow margin between too high a dosage of γ-butyrobetaine, the effect of which could not be reversed by carnitine in any amount, and too small an amount to have any significant action. Similarly, it was shown that carnitine itself was toxic when given in concentrations which were even lower than the toxic concentrations of γ-butyrobetaine.

The significant result of this study is the demonstration of a physiological role of carnitine in a system which normally functions in the absence of added carnitine. Despite the greater inhibition by γ-butyrobetaine in earlier blastoderms, it cannot be concluded that carnitine is without a role in physiological functions other than those involving growth. Further experiments should be designed to assay the requirements for L-carnitine in various adult organisms including man. A possible influence of other dietary factors on the biosynthesis of this material should also be investigated.

AMINO ACID METABOLISM IN PROTEIN DEFICIENCY

Protein deficiency in the experimental animal can be expected to manifest itself in a great many ways because of the many functions of proteins in enzymatic and other vital systems (*Nutrition Reviews* **7,** *298, 300 (1949);* **8,** *295 (1950);* **9,** *206 (1951);* **10,** *74 (1952)*). However, certain symptoms may be expected to appear first on the protein deficient diet either because a system requires a rapidly renewed source of protein, because its malfunctioning results in a metabolic disturbance most quickly, or possibly merely because the associated symptoms are most easily observed. It might be suspected that some of the first manifestations of deficiency would be in protein metabolism itself. That this may indeed be the case was considered by S. Kaplansky, N. N. Berezovskaya and Zh. Schmerling (*Biokhimiya* **10,** *401 (1945)*), who reported that protein deficiency in rats resulted in deranged transamination, but that amino acid synthesis was normal.

In a more recent paper, Berezovskaya and N. P. Smirnova (*Ibid.* **21,** *457 (1956)*) reported a reinvestigation of the problem, based on reports that protein synthesis was also subnormal in protein deficiency.

For this study, rats which had been on a diet containing only 3 per cent protein for three months, and which had the usual symptoms such as weight loss, hypoproteinemia, and low nitrogen excretion, were used. Their livers were prepared as slices for studies of synthesis and as homogenates for the study of transamination. When the slices were incubated with pyruvic, oxalacetic or alpha ketoglutaric acids, amino acid synthesis was generally depressed under that of the control animals, and in particular conversions of pyruvate to alanine and ketoglutarate to glutamic acid were reduced by about 50 per cent.

In experiments with the homogenates, ketoglutarate-alanine transamination was detected by semiquantitative paper chromatography of the amino acids formed in the homogenates. In deficient animals transamination was depressed an average of 30 per cent under the control rats, the amount of depression being correlated with the decrease in serum protein. It was also found that not all the derangement occurred at the same time, the ketoglutarate-alanine depression starting at four to seven days and the ketoglutarate-aspartate depression occurring much later. After two to three months on the deficient diet, synthesis and transamination in all systems were depressed.

Another early sign of protein deficiency is a disturbed oxidation of tyrosine and phenylalanine (S. Ya. Kaplansky and S. I. Kaplanskaya, *Biokhimiya* **14,** *130 (1949)*). As possible mechanisms for this effect, the authors considered an ascorbic acid deficiency, since this vitamin is known to be involved in tyrosine oxidation at the stage of oxidation of phenylpyruvic acid to 2,5-dihydroxyphenylpyruvic (*Nutrition Reviews* **11,** *17, 141 (1953)*). Also considered was a deficiency of glutathione, which may also be involved in the oxidation.

In an attempt to elucidate this effect, V. S. Kazantseva and S. Ya. Kaplansky (*Biokhimiya* **21,** *548 (1956)*) incubated liver slices or homogenates from protein deficient rats (as described by Berezovskaya and Smirnova, *loc. cit.*) with tyrosine, and analyzed for the unoxidized tyrosine. Tyrosine oxdiation in the deficient animals was only about 25 to 30 per cent of that in the control animals. However, neither ascorbic acid nor glutathione had any appreciable effect on the oxidation, despite the fact that in the deficient animals the liver ascorbic acid content was only about 30 per cent of the control values. However, the knowledge that transamination is inhibited in protein deficiency prompted the authors to try additions of ketoglutarate. This supplement brought the tyrosine oxidation by the deficient livers almost to normal even without

additional ascorbic acid, although the latter did give a further slight increase.

It thus appears that the inhibition of tyrosine oxidation in protein deficiency is primarily at the first step, the conversion of tyrosine to hydroxyphenylpyruvic acid, in which ketoglutarate transaminase is involved (*Nutrition Reviews* **10,** *92 (1952)*). A secondary deficiency in ascorbic acid is also apparent, but the enzymes involved in all the steps are evidently not critically affected.

Since the concentration of ketoglutarate was found to be at least normal in the deficient livers, the authors assumed that the rate of ketoglutarate formation is the important factor involved in tyrosine oxidation. As evidence for this idea it was found that although citric acid increased tyrosine oxidation in livers from normal animals, it had no effect on the livers of deficient animals. Thus, the conversion of citric acid to ketoglutarate was shown to be inadequate, and the basic disturbance was evidently concerned with the citric acid cycle.

If, as seems likely in the light of the present experiments, the disturbance in tyrosine oxidation in protein deficiency has its origin in so basic a mechanism as the defective operation of the citric acid cycle, it is probable that other symptoms of the deficiency will also be found to stem from this cause.

EFFECT OF MALEIC HYDRAZIDE ON RAT LIVER

The multitude of pesticides and food additives currently in use and proposed for future use poses a continuing problem for agencies such as the Food and Drug Administration, whose responsibility it is to assure the public of protection against harmful food contaminants and components. Among the many compounds that may find their way into food products is the growth regulator, maleic hydrazide, which is used as an antisprouting agent for potatoes. This compound, sprayed on the plants before harvest, is translocated to the tubers and prevents sprouting during storage.

Its effectiveness is considered to depend upon its ability to break chromosomes during mitosis, a property common to a large number of carcinogens (Darlington and McLeish *Nature* **167,** *407 (1951)*). Although the compound appeared to have a low order of toxicity for animals, the question of carcinogenicity was unanswered. Hence, W. A. Mannell and H. C. Grice (*Canad. J. Biochem. Physiol.* **35,** *1233 (1957)*) undertook an investigation in which they compared the carcinogenic properties of maleic hydrazide with those of paradimethylaminoazobenzene (butter yellow, DAB), an established carcinogenic agent.

Since thioacetamide and DAB (two chemically dissimilar compounds which are hepatic carcinogens) produce both hypertrophy and hyperplasia in rat liver, long term tests were performed on young male rats to determine whether similar changes occurred in rats fed on diets containing maleic hydrazide. A control group was maintained on a commercial stock laboratory diet; the test groups received the same diet containing 2 per cent of maleic hydrazide or 0.06 per cent of DAB.

Six rats from each group were killed at six, ten, 20 and 26 weeks. A sample of each liver was taken for histological examination, another sample was taken for nucleic acid analyses and the nuclei were isolated from the remainder. Both the ribose nucleic acid (RNA) and the desoxyribose nucleic acid (DNA) content of the livers were estimated. The nucleic acids were separated and the phosphorus content of each fraction was determined. The liver cell nuclei were isolated by differential centrifugation, the nuclei were resuspended and counted, and

the DNA content of the suspension was determined so that the DNA per nucleus could be calculated.

The average rate of gain and the average liver weight of rats receiving maleic hydrazide were not significantly different from the control values, but those receiving DAB grew more slowly and had higher relative liver weights (liver weight per unit of body weight), particularly after 20 and 26 weeks.

The results of the nucleic acid analyses showed a similar pattern. There was a significant increase in the DNA content of the livers of rats receiving DAB for 20 and 26 weeks. This was not due to an increase in DNA content per nucleus but to an increase in the number of cells and a decrease in tissue mass per unit of DNA. There was also a decrease in RNA per unit of DNA. In contrast, rats receiving maleic hydrazide could not be distinguished from control animals on the basis of the various nucleic acid analyses.

The pathological findings showed the same general pattern as the chemical analyses. The livers of rats receiving 2 per cent of maleic hydrazide for 26 weeks could not be distinguished from those of the control rats, and no neoplasms were observed in the livers of any of the 52 rats in the control and maleic hydrazide-treated groups. Livers from rats that had received DAB for six weeks did not contain neoplasms, but did show definite histological changes. At ten weeks tumors could be detected microscopically in the livers of the rats that received DAB. At 20 weeks tumors were readily visible, and by 26 weeks these were larger and more numerous.

The observations on the effect of DAB on liver nucleic acid content are in agreement with previous observations (Thomson *et al.*, *Biochem. J.* **53,** *460 (1953)*), and it appears that the changes noted serve as a reliable chemical indicator of neoplastic changes. The lack of effect of maleic hydrazide when it was fed at 30 times the level used for DAB, together with the similar observations on its non-carcinogenicity by Barnes *et al.* (*Nature* **180,** *62 (1957)*), lead to the conclusion that maleic hydrazide is non-carcinogenic.

In the testing of a compound for toxic effects, the decision as to what is adequate evidence of non-toxicity is a difficult one. How long should tests be continued? How many criteria should be used? How many species should be examined? How should the compound be administered? What type of controls are most suitable? There is almost no limit to the extent and variety of testing that can be undertaken in examining a single compound, let alone the multiplicity of new compounds that must be tested with the current rate of pharmaceutical and agricultural development. It is certainly encouraging to see that tests as thorough and as fundamentally sound as this are being performed.

ASCORBIC ACID AND COLLAGEN FORMATION

Although it is well known that a deficiency of ascorbic acid causes delayed and defective wound healing (*Nutrition Reviews* **4,** *331 (1946);* **10,** *124 (1952)*) and that the tissue formed during healing contains only a small amount of collagen (*Ibid.* **11,** *82 (1953);* **14,** *152 (1956);* **15,** *114 (1957)*), relatively little is known about the nature of the fundamental defect that prevents the formation of normal repair tissue in scorbutic animals (Robertson and Hinds, *J. Biol. Chem.* **221,** *791 (1956);* Bowness, *Brit. J. Nutrition* **11,** *152 (1957)*).

The collagen molecule, an essential component of normal repair tissue, contains a high percentage of hydroxyproline which is derived from proline (M. R. Stetten, *J. Biol. Chem.* **181,** *31 (1949)*). Also it has

been shown that fibroblast preparations obtained from guinea pigs have the ability to convert proline to hydroxyproline (Smith and Jackson, *Biochem. J.* **64,** *8P (1956)*). Since ascorbic acid has been implicated in hydroxylation reactions involving a number of aromatic compounds (Udenfriend, *Amino Acid Metabolism* Ed. McElroy and Glass *The Johns Hopkins Press, Baltimore p. 881, (1955)*), it is a logical extension of these observations to study simultaneously the influence of ascorbic acid on the hydroxylation of proline and on the synthesis of collagen. Such a study undertaken by B. S. Gould and J. F. Woessner (*J. Biol. Chem.* **226,** *289 (1957)*) has produced some interesting observations concerning the role of ascorbic acid in wound healing.

Normal and scorbutic guinea pigs (250–300 g.) were the subjects in these *in vivo* experiments. Circles of skin 1.6 cm. in diameter were removed from the intrascapular region in order to study the effect of ascorbic acid on wound healing. The wounded areas were carefully marked and the repair tissue was collected at various intervals during depletion or during supplementation with ascorbic acid.

The repair tissue was minced in acetone to remove moisture, then extracted with ether to remove fat. The extracted tissue was suspended in a small volume of water and heated in an autoclave to convert the collagen to soluble gelatin. The gelatin thus extracted was collected and dried. After hydrolysis of the dried material with acid the concentrations of hydroxyproline, proline and glycine were estimated by chemical methods.

Since hydroxyproline is assumed to occur only in collagen, and since collagen contains 13.4 per cent of hydroxyproline, the hydroxyproline content of the tissue multiplied by 7.46 was taken as a measure of the amount of collagen present.

The first experiments consisted of a time study in which the influence of ascorbic acid on the rate of collagen formation was determined by analyzing for hydroxyproline in repair tissue taken from scorbutic guinea pigs at intervals after wounding. Skin circles were removed from the animals after they had been fed a diet deficient in ascorbic acid for seven or 14 days. The hydroxyproline content of repair tissue from animals that that received no ascorbic acid was very low and remained low during the 16 days following wounding.

When ascorbic acid (30 mg. per day) was administered immediately after wounding, and daily thereafter, little hydroxyproline was formed during the six days following wounding even though there was histological evidence of cellular organization. Between six and eight days, however, there was a rapid increase in the hydroxyproline concentration of the repair tissue and this continued for eight days until the estimated collagen content of the repair tissue had risen, from less than 4 per cent, to 20 per cent. When ascorbic acid supplementation was delayed for 12 days after wounding there was very little lag in collagen formation, and within 48 hours after ascorbic acid administration the hydroxyproline content of the repair tissue had risen to the level found only after eight days in tissue from the positive control group. These results are similar to results obtained by Dunphy and Udupa (*Nutrition Reviews* **14,** *152 (1956)*). Both groups of workers interpreted their findings as evidence for the formation of a non-fibrous collagen precursor which, only in the presence of an adequate supply of ascorbic acid, can be converted into fibrous collagen.

Gould and Woessner (*loc. cit.*), in another series of experiments, wounded animals at intervals after ascorbic acid had been withdrawn from the diet and analyzed the repair tissue for hydroxyproline 12 days after wounding. The hydroxyproline content of repair tissue from animals wounded within two days after ascorbic acid withdrawal was normal. If wounding was performed four days after ascorbic acid withdrawal much

less hydroxyproline was present in the repair tissue and, if the delay was seven days or more no evidence of hydroxyproline formation was obtained.

On the assumption that a collagen precursor, having properties similar to collagen, might accumulate in granulation tissue formed by animals lacking ascorbic acid, Gould and Woessner extracted granulation tissue from wounds in animals maintained on scorbutic diets for 12 days after wounding. They analyzed the extracts for proline, glycine and hydroxyproline. Similar experiments were performed on animals which were maintained on the ascorbic acid deficient diet for 12 days and were then given 30 or 50 mg. of ascorbic acid for three days before collection of the repair tissue.

On the basis of average analyses, the ratio of hydroxyproline to proline to glycine, of the extracted collagen, should be 1:1.25:2.0 and any substantial deviation from this ratio might be taken as evidence of the presence of a collagen precursor differing in amino acid composition from collagen. The ratio of hydroxyproline to proline to glycine, for material extracted from repair tissue of scorbutic animals, was 1:3.9:4.5. However, when ascorbic acid was administered to the deficient animals for just three days prior to sampling, the ratio was 1:1.6:2.2. Measurement of the absolute amounts of each of the amino acids in a few samples indicated clearly that the restoration of the ratio toward the theoretical value was a result of a decrease in proline content and an increase in hydroxyproline content.

Determination of free proline in wound tissue from both normal and scorbutic guinea pigs indicated that less than 2 per cent of the proline was in the free form. It seems evident, therefore, that the proline was incorporated into a high molecular weight collagen precursor, and that hydroxylation of bound proline occurred.

These observations represent a substantial contribution toward an understanding of the nature of the processes involved in collagen formation and wound healing and may lead eventually to an understanding of a specific function of ascorbic acid and to an explanation of the molecular basis for certain deficiency signs in scorbutic animals. Since Smith and Jackson (*loc. cit.*) have been able to demonstrate the conversion of proline to hydroxyproline *in vitro* in fibroblast preparations obtained from guinea pigs injected subcutaneously with carageenin, it should now be possible to study, in detail, the mechanism of proline hydroxylation and collagen formation.

BIOLOGICAL VALUE OF PROTEINS

W. Lintzel (*Deutsche med. Wochenschrift* **80,** *1047 (1955)*) has claimed that plant proteins are more efficient than animal proteins in maintaining the nitrogen balance in sedentary, fully grown human subjects. According to this author, animal proteins, which are relatively richer in essential amino acids (lysine, valine, trypotophane, histidine, phenylalanine, leucine, isoleucine, threonine, arginine and methionine), as compared to plant proteins, are required more specifically for growth, synthetic and regeneration processes. In his studies, Lintzel (*loc. cit.*) employed a technique which consisted of (1) preconditioning his subjects by a short period of protein fasting so that a nearly perfect nitrogen balance was attained, (2) substituting similar amounts of various proteins in the diet and (3) analyzing the urine and feces for nitrogen during a six- to nine-day period.

With this method, he determined that only 0.48 g. of protein from potatoes per kilo of body weight, and 0.52 g. of protein from rye grain per kilogram of body weight, were required to maintain nitrogen balance in

adults, whereas 0.66 g. of proteins from milk, 0.70 g. of protein from meat, and 0.72 g. of protein from eggs, were required for this purpose. Of the plant proteins tested by this technique, only that of kidney beans was required in greater amounts than the animal proteins listed above.

Such claims, so late in the development of the essential amino acids concept (*Nutrition Reviews* **15,** *323, 337 (1957);* **16,** *22, 56 (1958)*), have, of course, provoked considerable protest. The procedure used by Lintzel (*loc. cit.*) has been attacked on several grounds, including the following: (1) The subjects were given too high a proportion of fat in the diet; (2) in the early course of the experiments the subjects suffered a nitrogen deficiency which influenced the remainder of the research period; (3) the period of observation was much too short; and (4) the subjects were losing weight during the course of the experiment.

E. Kofranyi (*Z. f. physiol. Chem.* **309,** *253 (1957)*) has attempted to correct a number of the deficiencies of the method originated by Lintzel (*loc. cit.*) and has obtained completely different results. For a large part of this work a single male subject was used (age 48, height 169 centimeters, weight 75.4 Kg.). The total period of nutritional testing was divided into three phases. During the first interval (Period A), milk was the sole source of protein in the diet. This period lasted for 56 days. Prior to the beginning of the next experimental diet, the subject was given 15 days of vacation, during which he ate what he desired. Period B lasted for 62 days and the only protein provided during this interval was that present in rye bread. Following this test, the subject was again allowed a period of free diet (12 days). During the third period (C), which had a duration of 14 days, no protein was present in the food supplied. In all experimental periods the total daily calories amounted to approximately 3000 calories, and in the first and second nutritional periods the total amounts of protein administered per day were almost identical. Sodium glutamate was included in the diets toward the ends of Periods A and B. Pooled diets, urine and feces were analyzed for nitrogen, phosphorus and calcium content at seven-day intervals, and the nitrogen balance was determined from these results.

It was found that the biological value of protein from rye was 22 per cent lower than that of milk protein. The addition of glutamic acid did not influence the biological value of the plant protein while that of milk protein was slightly reduced. Substitution of one-fifth of the total protein in a pure vegetable diet with cheese protein resulted in a 39 per cent enhancement of the biological value in terms of nitrogen balance.

Although these results were obtained mainly from a single subject, they lend considerable support to the notion that animal proteins are essential for maintenance of the adult organism as well as for growth, regeneration and synthesis. However, cells of the sedentary adult are certainly involved in some regeneration and synthesis. It is quite possible that the animal proteins (with their balance of essential amino acids) are necessary to support these latter two processes of the adult, whereas they may not be specifically required for cellular maintenance.

The studies in which glutamic acid and proteins from cheese were substituted into the diets further support the conclusions of J. B. Allison (*Nutrition Reviews* **14,** *129 (1956)*) and others, who have stated that a balance between essential and non-essential amino acids is the important factor in the utilization of a protein by the organism. An example of a vegetable protein which apparently fails utterly in this respect, at least in the growing animal, is the protein from the cultured mushroom *Psalliota bispora*. H. Fink and H. Kubin (*Z. f. physiol. Chem.* **309,** *158 (1957)*) have shown that the administration to growing rats of an otherwise adequate diet, containing 92 per cent of

the protein in the form of culture mushroom substance, causes polyneuritic convulsions followed by death within 5 to 20 days. Earlier studies had indicated a high incidence of liver necrosis in growing rats fed various proportions of this protein material. Since polyneuritic convulsions have been reported in cases of starvation, and since the authors were able to alleviate the convulsions with vitamin B_1 (the vitamin has been shown to be somewhat effective in preventing the polyneuritic convulsions associated with starvation) they have tentatively concluded that the mushroom protein is not poisonous *per se*, but merely fails to provide the proper amino acids essential for structural growth.

PHOSPHATE ACTIVITY IN WHITE CELLS

Histochemical techniques make it possible to determine alkaline phosphatase activity in the white cells. M. Wachstein (*J. Lab. & Clin. Med.* **31,** *1 (1946)*) demonstrated that the alkaline phosphatase activity of the white cells is almost completely confined to the mature segmented neutrophils. Normal polymorphonuclear leucocytes contain only small amounts of this enzyme, but the activity increases considerably in acute infections and after trauma, hemorrhage, or infarction. In chronic myeloid leukemia, Wachstein found little or none of this enzymatic activity in the white cells.

Recently, B. J. Leonard, M. C. G. Isaels and J. F. Wilkinson (*Lancet* **I,** *289 (1958)*) have reported a modification of the original histochemical technique, which offers a simple method of determining the alkaline phosphatase activity in the blood or bone marrow smears. By this technique, the alkaline phosphatase activity appears to be black granules in the cytoplasm of the neutrophil polymorphs. These investigators noted that in smears obtained from 50 normal individuals examined, an average of only one per cent of the neutrophils had strong phosphatase activity, and 18 per cent had weak activity. No activity was noted in an average of eight per cent of the cells. In 50 patients with myeloid leukemia an average of one per cent of the neutrophil polymorphs showed strong phosphatase activity and a mean value of six per cent showed weak activity. No phosphatase activity was shown by an average of 92 per cent of the cells. Patients with polycythemia vera (50) had 65 per cent of the white cells showing strong phosphatase activity, 32 per cent showing weak phosphatase activity, and 3 per cent showing none. In contrast to patients with chronic myeloid leukemia, the phosphatase activity in the white cell obtained from 17 patients with leukemoid reactions had 60 per cent showing activity, 34 per cent showing weak activity and only 6 per cent showing no activity.

The 17 cases of leukemoid reactions were associated with the following disorders: Hodgkin's disease, myelosclerosis, carcinomatosis, tuberculosis, acquired hemolytic anemia and reticulosarcoma. In all these cases, by the ordinary histological staining methods, the cells appeared to be identical with those found in myeloid leukemia. The phosphatase activities were distinctively different, however. Rather than having a large number of cells having no alkaline phosphatase activity and only a few showing strong activity, as is noted in white cells from patients with chronic myeloid leukemia, the reverse pattern of phosphatase activity was noted in individuals with the leukemoid reaction. Thus, these investigators believed that the staining for phosphatase activity occasionally offers the only means of differentiating between these two disease groups.

In one case with acute lymphatic leukemia the patient had 82 per cent of the neutrophils in a smear showing strongly positive reaction, and only four per cent with no activity. Following a series of

treatments using cortisone, there was a complete clinical remission although there was little change in the phosphatase activity of the white cells. The patient died of leukemia three months later.

The role of the phosphatase in the metabolism of the white cell is not known at present, although it has been noted that a modification in the alkaline phosphatase activity in the cells of patients with chronic myeloid leukemia appears to precede the development of the definite cytological abnormality. Since there is an increase in the alkaline phosphatase activity in the white cell following various types of stress (*e.g.* hemorrhage, infection, trauma, and the like), it has been suggested that this may also be another effect controlled by the pituitary-adrenal glands. Also, the administration of cortisone or ACTH has been noted to increase the phosphatase activity of the white cells. Should adrenal activity be reduced (such as in Addison's disease) the administration of cortisone will increase the phosphatase activity of the white cells, but ACTH will not. However, as yet there has been no definitive evidence to indicate that there is a definite control of the white cell alkaline phosphatase activity by either the adrenal or pituitary glands.

The addition of the dynamic concepts of enzyme activity with the well studied histological patterns of disease has, in this case, offered much more information concerning the disease than either technique alone.

Notes

UPTAKE OF RADIOACTIVE TRYPTOPHAN BY MALIGNANT CARCINOID TUMOR

Certain tumors in the intestine produce large quantities of 5-hydroxytryptamine (serotonin). A syndrome occurs in these patients which is characterized by diarrhea, cutaneous flushing, respiratory distress and valvular disease of the heart. The relationship between these malignant tumors and serotonin production has been reviewed earlier (*Nutrition Reviews* **15,** *156 (1957)*).

The precursor of serotonin is the essential amino acid, tryptophan and S. Udenfriend and E. Titus (*Amino Acid Metabolism, Johns Hopkins Press, Baltimore, p. 945, (1955)*) have reported that giving extra tryptophan to patients with carcinoid tumors causes an increase in the urinary excretion of 5-hydroxyindolacetic acid. The latter acid is a metabolic product derived from serotonin. In addition, Udenfriend, H. Weissbach, and S. Sjordsma (*Science* **123,** *669 (1956)*) have found that the administration of tryptophan labeled with radioactive carbon to patients with carcinoid tumors caused an increase in the radioactivity of the 5-hydroxyindolacetic acid which was excreted in the urine. These studies would indicate that the radioactive tryptophan gave rise to the tagged serotonin which was in turn metabolized and excreted as indolacetic acid.

Recently J. W. Cole and L. Matthews (*Surg. Forum* **8,** *191 (1958)*) have reported the administration of tryptophan tagged with radioactive carbon to a patient with a malignant carcinoid tumor five hours before operation. During the surgical procedure to relieve a partial intestinal obstruction, biopsies of the metastatic carcinoid tumor, normal liver, skeletal muscle and small intestinal mucosa were obtained. The tissue biopsies were oxidized and the resultant carbon dioxide assayed for the C^{14} content, utilizing a flow-type Geiger counter. These investigators reported that the skeletal

muscle and liver showed 1.5 and 6.8 counts per minute per mg. of dry weight, but the carcinoid tumor yielded 10.2 counts per minute per mg. of dry tissue. These investigators suggest that the administered tryptophan is taken up by the tumor and probably gives rise to the serotonin present in the tumor. It was also found that the normal intestinal mucosa yielded 29 counts per minute per mg. of dry tissue. In this respect it should be noted that the patient was operated upon for relief of a partial small bowel obstruction. Thus, one might expect a poor absorption of the administered tryptophan and consequently an increased amount in the intestine.

This study would indicate that there is a direct uptake of orally administered tagged tryptophan by the metastatic carcinoid tumor of this patient and suggests that these tumors are utilizing tryptophan for the endogenous production of 5-hydroxytryptamine.

Letter to the Editor

Sir:

The review of our investigation on the nutritive value of bacterial cells (*Nutrition Reviews* **16,** *115 (1958)*) was well done. Lest the reader of the review be misguided, however, it is pertinent to point out that we did not define biological value in our paper, and had we done so, we would have written that biological value is the percentage of *absorbed* protein that is *utilized* by the animal for purpose of gain (retention) and maintenance (replacement of endogenous and metabolic losses).

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Recent Nutrition Books

Calorie Requirements (FAO Nutritional Studies, No. 15). Report of the Second Committee on Calorie Requirements. Food & Agricultural Organization of the United Nations, Rome, Italy. 1957. Pp. 67.

Methods of Biochemical Analysis. VI. David Glick, Editor. Interscience Publishers, Inc., 250 Fifth Ave., New York 1, N. Y. Pp. 317.

Chemistry of Lipides as Related to Atherosclerosis. Irvine H. Page. Charles C. Thomas, Springfield, Illinois. Pp. 342.

General Biochemistry. Joseph S. Fruton and Sofia Simmonds. John Wiley & Sons, Inc. Pp. 1077. Price: $18.00.

Cholesterol as Related to Atherosclerosis: A Review of the Literature, 1950 to July 1957. Helen I. Mattill. Cereal Institute, Inc., 135 South LaSalle St., Chicago 3, Illinois.

Role of Dietary Fat in Human Health: A report of the Food & Nutrition Board. National Academy of Sciences-National Research Council. Prepared by the Committee on Fats in Human Nutrition. 1958. Pp. 32. Price: $1.00.

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